REVIEW ARTICLE

Role of autophagy in neurodegenerative diseases

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The growing range of implications of autophagy in a myriad of human physiological and pathological conditions has witnessed an exponential increase in the number of studies published over the last decade. The role of autophagy to function predominantly as a cellular survival mechanism has been widely accepted over the last few years. It is an evolutionarily conserved protein degradation pathway for long-lived proteins and organelles, which contributes to tissue and energy homeostasis. Dysfunction of this process is associated with diverse human diseases, ranging from cancer, infectious diseases and myopathies to neurodegenerative diseases. This review focuses on the role of autophagy in neurodegenerative diseases, where in most instances the mutant aggregate-prone proteins are autophagy substrates. Some of these mutant proteins can impair autophagy and augment neurodegeneration. Stimulation of autophagy by chemical inducers enhances autophagic degradation of aggregate-prone proteins and protects against neurodegeneration in several models of neurodegenerative diseases. The small molecule autophagy enhancers are of paramount importance for future therapeutic studies in other disease conditions beyond neurodegeneration, and also offer great potential in the study of signalling pathways regulating autophagy.

Keywords: Aggregate-prone proteins, autophagy, neurodegenerative disease, small molecules.

Autophagy as a cell survival process

OVER the last decade, the field of autophagy has witnessed an exponential growth in its application in various biological aspects as evident from the number of papers published (Figure 1 *a*). This is largely due to its growing range of implications in a myriad of human physiological and pathological conditions, such as, but not limited to, development, immunity, longevity, cancer and neurodegeneration[1,2] (Figure 1 *b*). Although initially described as programmed cell death type II, the concept of autophagy has evolved over the years from a cell-death phenomenon to a cell-survival process. The role of autophagy to function predominantly as a cellular survival mechanism has been widely accepted thus far, whereas autophagic cell death has been reported only in certain contexts[3,4].

Autophagy is an evolutionarily conserved process from yeast to mammals. Whereas the morphological characteristics of autophagy have been first described in mammalian cells, its molecular components are well characterized in yeast, wherein the fundamental discoveries pertaining to the identification of *Atg* (autophagy-related) genes were made by Yoshinori Ohsumi's group in the early 1990s. Thereafter, genetic analyses in yeast have unravelled a number of *Atg* genes, many of which have been shown to have mammalian orthologs[5]. Initial studies in yeast mutants have uncovered distinct steps of the molecular machinery underlying autophagy, details of which are reviewed elsewhere[6,7]. A brief history of autophagy is summarized in Box 1.

Autophagy occurs in all tissues and is normally induced by starvation. It is a cellular degradation pathway for long-lived proteins and cytoplasmic organelles. During autophagy, a double-membrane structure called phagophore arises in the cytoplasm, whose origin has been recently reported to be from the endoplasmic reticulum[8,9], plasma membrane[10] and mitochondria[11]. The expanding phagophore non-selectively engulfs cytoplasmic components to form a double-membrane vesicle called autophagosome, which then fuses with the lysosome to form the degradative autolysosome (Figure 1 *b*). The engulfed cargo is degraded by the acidic lysosomal enzymes to generate energy and building blocks for protein synthesis during the nutrient-limitation condition. Upon restoration of the nutrients, autophagy is turned-off. However, autophagy can also be selective in some instances; for example, in the context of many neurodegenerative diseases for the clearance of aggregate-prone proteins, or for the removal of certain invading pathogens in case of infectious diseases[2,12–14]. Terminologies related to the process of autophagy are listed in Box 2.

Basal autophagy exists in all cells of the living body for the removal of naturally occurring misfolded proteins, thereby having a housekeeping role and acting as a crucial player in tissue homeostasis[15]. For instance, tissue-specific deletion of key autophagy genes in mice muscle, heart or central nervous system has resulted in muscular atrophy, cardiac hypertrophy and neurodegeneration, respectively[16–19]. Additionally, knockout of an essential autophagy gene *Atg5* in mice causes lethality soon after

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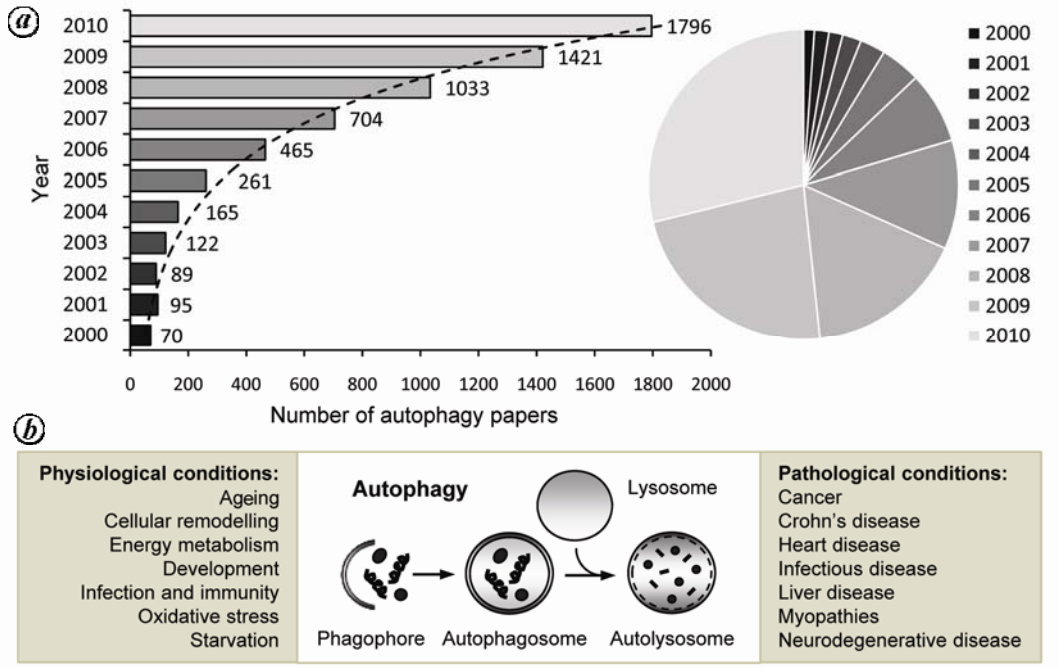

Figure 1. Growing implications of autophagy in human physiological and pathological conditions. ***a***, Exponential growth in the number of papers published in the field of autophagy during 2000–2010 (source: Pubmed). ***b***, Schematic representation of the autophagy pathway and its implications in various human physiological and disease conditions.

Box 1. Brief history of autophagy.

Christian de Duve coined the term autophagy in 1963, after his initial discovery of the lysosome in 1955. The period between the 1960s and 1980s was associated with the morphological analysis of mammalian autophagy. These include studies on the terminal stages of autophagy by Christian de Duve and others, as well as the early and intermediate steps involving the identification of phagophore by Per Seglen. In 1992, Yoshinori Ohsumi showed that the process of autophagy in yeast is similar to that in mammals, and thereby suggested yeast as a model system for genetic studies. Subsequently, Ohsumi's group carried out the first genetic screen in yeast for autophagy mutants. This was followed up with similar screens by other groups to identify *Atg* (autophagy-related) genes in yeast. The target of rapamycin (*Tor*) gene was isolated from yeast in 1993 and from mammals in 1994. In 1995, Alfred Meijer showed that rapamycin, an inhibitor of TOR, induces autophagy. Noboru Mizushima identified the first mammalian autophagy genes, *Atg5* and *Atg12*, in 1998, and together with Tamotsu Yoshimori in 2000, identified the mammalian homologue of yeast Atg8, MAP1LC3 (also known as LC3). This led to robust development of LC3-based assays for monitoring mammalian autophagy. Over the last decade till date, autophagy is gaining recognition as an important area of study by many biologists due to its growing implications in diverse human physiological and pathological conditions.

birth, suggesting a role of autophagy during the early neonatal starvation period by contributing to the maintenance of energy homeostasis[20].

The only known mammalian protein that specifically associates with the autophagosome membrane throughout their lifespan is the microtubule-associated protein (MAP) light chain 3 (LC3), which is the yeast ortholog of *Atg8*. LC3 is post-translationally modified into the LC3-I form, which is cytoplasmic, whereas it conjugates to phosphatidylethanolamine upon induction of autophagy to form the autophagosome-associated LC3-II form[21]. The levels of LC3-II directly correlate with autophagosome numbers. Although LC3-based assays are used to measure autophagy, it is critical to monitor autophagic flux through the entire pathway instead of the steady-state levels of LC3-II. Assays to monitor autophagy accurately in mammalian systems have been established with tandem fluorescent-tagged LC3 marker or analysis of the clearance of specific autophagy substrates[22–27].

The classical pathway regulating mammalian autophagy involves the serine/threonine kinase, mammalian target of rapamycin (mTOR), in which autophagy is negatively regulated by the mTOR complex 1 (mTORC1)[1,28,29]. The activity of mTORC1 can be inhibited by rapamycin or starvation, which are well-established inducers of autophagy[29] (Figure 2). Many diverse signals, such as growth factors, amino acids and

Box 2. Glossary of terminologies.

Autolysosome: This is formed in the cytoplasm by the fusion of an autophagosome with a lysosome. It is also referred to as a degradative autophagic vacuole, AVd. It normally has an acidic pH and consists of proteolytic enzymes which degrade the components delivered by the autophagosome. Following degradation of the autophagic cargo, autolysosome becomes a lysosome or a residual body.

Autophagosome: This is a double-membrane vesicle originating in the cytoplasm from an isolation membrane called phagophore. It is also referred to as an initial autophagic vacuole, AVi. The autophagosome contains engulfed cytoplasmic components, such as long-lived proteins, protein complexes and inclusions, as well as organelles, but lacks proteolytic enzymes.

Autophagy: Macroautophagy, which is generally referred to as autophagy, is an ubiquituous process of protein degradation within the lysosome in eukaryotic cells. The term autophagy means 'eating' (phagy) of part of the cells' self (auto). This process initiates with the formation of a phagophore followed by engulfment of cytoplasmic cargo in completed autophagosome, which then fuses with a lysosome to form an autolysosome where the contents are degraded. Following degradation of the intracellular components, the macromolecular constituents are recycled. Autophagy is generally non-selective, but can be specific in certain contexts.

Lysosome: This is a cytoplasmic degradative organelle found in higher eukaryotes that has a highly acidic pH and contains various proteolytic enzymes, such as cathepsins.

Phagophore: This is a double-membrane cytoplasmic structure suggested to be arising from the plasma membrane, endoplasmic reticulum or mitochondria. It is also referred to as an isolation membrane and is the initial sequestering compartment of autophagy that leads to the formation of autophagosomes.

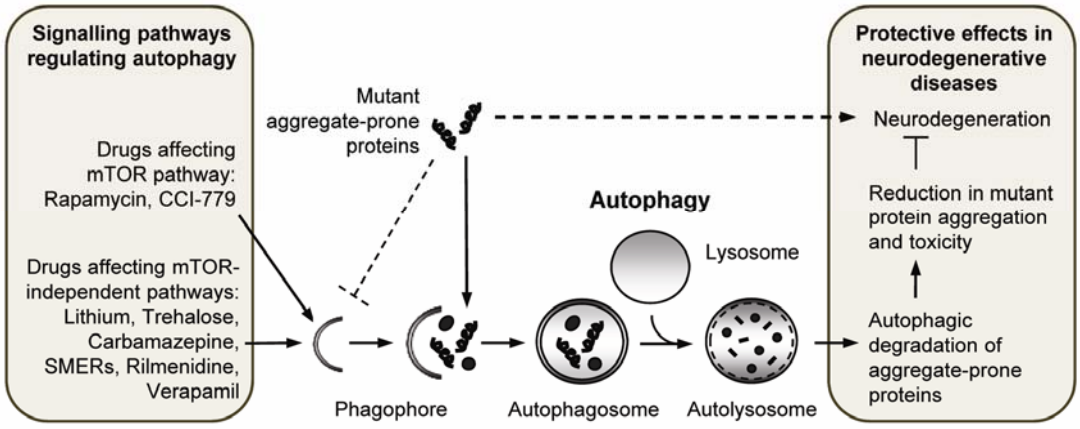

Figure 2. Protective role of autophagy in neurodegenerative diseases. Autophagy can be regulated by both mTOR-dependent and mTOR-independent signalling pathways, which are amenable to perturbations by small molecules. It is a major degradation pathway for the clearance of neurodegeneration-associated aggregate-prone proteins. In certain instances, the mutant proteins can impair the autophagy pathway and augment neurodegeneration. Stimulation of autophagy by chemical inducers enhances autophagic degradation of these mutant proteins and rescue against neurodegeneration in several models of neurodegenerative diseases. Adapted from Renna *et al.*[62].

energy status regulate autophagy by the mTORC1 pathway[30]. Autophagy can also be regulated independent of mTOR[13] (described later in this review).

Autophagy dysfunction contributes to neurodegeneration

Protein misfolding disorders or proteinopathies encompass a family of diverse human neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), prion diseases, and polyglutamine expansion disorders, including Huntington's disease (HD) and various spinocerebellar ataxias (SCAs)[31–33]. The hallmark of this category of diseases is the presence of intracellular mutant protein aggregates (also referred to as inclusions) in the neurons. However, extracellular amyloid plaques are seen in AD, whereas prion aggregation can occur both intracellularly and extracellularly[32]. The conformational change attributed to the mutant proteins is generally believed to promote the disease by a toxic gain-of-function.

A direct link between autophagy and neurodegeneration has been established by loss of basal autophagy in mouse brains through conditional knockout of key autophagy genes, *Atg5* or *Atg7*, which results in a neurodegeneration phenotype with the accumulation of ubiquitinated protein aggregates in the absence of any disease-causing proteins[18,19]. This suggests that autophagy is vital for cellular quality control in neurons for the turnover of proteins and organelles. A number of recent studies have highlighted a role for autophagy dysfunction as a contributing factor in various neurodegenerative diseases due to the accumulation of mutant aggregate-prone proteins (which are autophagy substrates; described in the next section), and thereby augmenting their toxicity. Neurodegeneration-associated mutations in presenilin 1 (AD)[34], huntingtin (HD)[35], α-synuclein, parkin, LRRK2, PINK1 (PD)[36–39], dyenin, ESCRT-III (ALS)[40,41] and laforin (Lafora disease)[42] have been shown to inhibit autophagy either at the synthesis or maturation stages of autophagosomes, or at the level of cargo recognition[43] (Figure 2). Therefore, stimulating autophagy for the removal of these toxic species may be beneficial in the context of neurodegenerative diseases.

Interestingly, the clearance of mitochondria by autophagy (termed mitophagy) has been recently shown to be regulated by parkin and PINK1, wherein parkin is recruited to the damaged mitochondria to facilitate its clearance via autophagy in a manner that is dependent on PINK1 stabilization on these organelles. Mutations in these proteins associated with familial PD disrupt parkin translocation and impair mitophagy, thereby leading to an accumulation of dysfunctional mitochondria[37,38]. In general, since mitochondria are autophagy substrates, an impairment of autophagy occurring in neurodegenerative conditions has implications in the underlying oxidative stress reported in such disease scenarios. Furthermore, excitotoxic stress arising due to Ca^{2+} overload may also contribute to neurodegeneration, in part, by autophagy dysfunction, since elevated Ca^{2+} impairs autophagy[13,44]. Moreover, inactivation of autophagy will also enhance the susceptibility of neurons to apoptotic insults[45,46].

Small molecule autophagy enhancers as therapeutic targets

Various mutant, cytoplasmic, aggregate-prone proteins associated with a number of neurodegenerative diseases have been shown to be degraded predominantly by autophagy (Figure 2); first demonstrated by David Rubinsztein's group. These include mutant huntingtin (HD), A53T and A30P α-synuclein mutants (PD), mutant superoxide dismutase 1 (ALS), ataxin 3 (SCA3), mutant tau (fronto-temporal dementia) and mutant prions (prion disease)[13,14,47]. The polyubiquitin-binding protein p62, also known as sequestosome 1 (SQSTM1) found in the protein aggregates in neurodegenerative diseases, is believed to be involved in linking the polyubiquitinated protein aggregates to the autophagic machinery via LC3 on the autophagosome membrane[48,49]. Since autophagy is a major degradation pathway for aggregate-prone proteins, the levels of these mutant proteins are elevated if autophagy is impaired; conversely, their levels are reduced if autophagy is stimulated by chemical or genetic means. Therefore, enhancing autophagy can be employed as a possible therapeutic strategy in neurodegenerative diseases where the mutant proteins are autophagy substrates[13,50].

Initial studies from David Rubinsztein's group using the mTOR inhibitor rapamycin, or its analogue the CCI-779, have shown protective effects in transgenic animal models of HD[51,52] (Figure 2). Thereafter, rapamycin was reported to have beneficial effects in a number of neurodegeneration models, including SCA3 mice[46,53,54]. Although these evidences are exciting, the use of mTOR inhibitors precludes long-term drug administration in neurodegenerative disease patients because mTOR has many vital cellular functions like translation and cell growth. Therefore, induction of autophagy independent of mTOR may provide a more rational treatment approach.

Further studies from the same group reported the first mTOR-independent autophagy pathway regulated by inositol and inositol 1,4,5-trisphosphate (IP_3) levels, in which autophagy could be induced by inositol-lowering agents like lithium and carbamazepine[55,56] (Figure 2). A chemical screen was employed to identify novel autophagy modulators, where a number of small-molecule enhancers of rapamycin (SMERs) and their analogues have been shown to induce autophagy independent of mTOR and rapamycin[57]. A subsequent screen with FDA-approved drugs identified a cyclic mTOR-independent autophagy pathway regulated by Ca^{2+}–calpain–$G_{s\alpha}$ and cAMP–Epac–PLC-ε–IP_3 pathways, in which multiple drug targets acting at distinct stages induce mTOR-independent autophagy[44]. A parallel screen with FDA-approved drugs also reported similar hits, such as the L-type Ca^{2+} channel antagonists[58]. One of the most potent mTOR-independent autophagy inducers is trehalose, which also acts as a chemical chaperone; thereby it can exert dual protective effects as an aggregation inhibitor and an autophagy inducer in the context of neurodegenerative diseases[59]. Furthermore, triggering autophagy via mTOR-dependent and mTOR-independent pathways has additive effects in enhancing autophagy and protection against neurodegeneration[60]. Many of these mTOR-independent autophagy inducers have been shown to have beneficial effects in cellular and transgenic animal models of various neurodegenerative disorders by enhancing the clearance of aggregate-prone proteins, and may therefore counteract the autophagy dysfunction reported in some of these diseases. Details of chemical inducers of autophagy are listed elsewhere[13,50,61–63].

Future perspectives

The autosomal-dominant proteinopathies that are potentially amenable to autophagy upregulation present an important opportunity for delaying the onset of disease. Remarkably, a clinical trial with lithium in ALS patients, as well as in mouse models, was found to increase survival and attenuate the disease progression[64]. Apart from the neuroprotective effects of lithium[65], this fascinating but preliminary result was attributed partly due to autophagy upregulation[55,64]. Interestingly, autophagy upregulation with chemical compounds was also shown to be protective in a range of disease models, including liver diseases, muscle diseases to infectious diseases, and also in longevity, where autophagy acts as a protective pathway[66–69]. Although autophagy has growing implications in several human pathological conditions, the chemical inducers of autophagy offer great potential for future therapeutic studies and could also be utilized in the study of intracellular signalling pathways regulating autophagy.

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